

B 4/ 54. The method of claim 52, wherein said synthetic tubular element has an inner cross-sectional area which is substantially equivalent to an inner cross-sectional area of a blood vessel.

55. The method of claim 54, wherein said inner cross-sectional area of said synthetic tubular element is about 7 to about 700 mm<sup>2</sup>.

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### REMARKS

Claims 1 – 12, 19 – 25 and 51 - 55 are presently before the examiner. Applicant has amended claims 1 – 9, 19, 20, 23 and 51 – 55 to more clearly point out that which applicant considers the invention. The amended claims are fully supported by the specification and add no new matter. A clean version of the amended claims is provided above. Addendum A is a "VERSION OF CLAIMS SHOWING CHANGES MADE" AND Addendum B is a "COMPLETE SET OF CLAIMS AS THEY WILL APPEAR ONCE THE AMENDMENTS HEREIN ARE ENTERED."

### Objection to the specification

The examiner has objected to the specification as, in the examiner's view, attempting to incorporate by reference essential material contained in a non-patent publication or a foreign patent or patent application. The specification has been amended to remove any attempt at incorporation of the references cited and to point out that such references are provided merely as an aid to the reader who would like to have more detailed information regarding the indicated subject matter. The literature references do not relate to essential material and are not necessary to the understanding or operation of the invention.

### 35 USC §112, second paragraph rejection of claims 1 – 12, 23 and 52 – 55

The examiner rejected claims 1 – 12, 23 and 52 – 55 under §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the

subject matter that applicant regards as the invention. In particular, the examiner believes that the use of the phrases “being genetically transformed” and “being coated” renders the claims unclear. In addition, the examiner is of the opinion that the word “and” in front of the words “bone marrow” in claim 23 also renders that claim ambiguous also.

#### **Applicant's response**

The claims have been amended, thereby rendering the rejection moot. The examiner is requested to withdraw the rejection.

#### **35 USC 102 rejection of claims 1 – 5, 7 – 12, 19 – 22, 24, 25 and 52 – 55**

The examiner rejected claims 1 – 5, 7 – 12, 19 – 22, 24, 25 and 52 – 55 under §102 as being anticipated by US Pat. No. 5,785,965 to Pratt, et al. The examiner notes that Pratt discloses a prosthetic vascular e-PTFE graft seeded with genetically transformed endothelial cells from the host or other sources (col. 3, lines 29 – 36.). The examiner also notes that, while Pratt does not teach cells specifically transformed to express an adherence factor, the cells do express adherence factors naturally and therefore meet the limitation. Applicant traverses.

#### **Applicant's response**

Applicant respectfully disagrees with the examiner's comment that any natural expression of an adherence factor by the endothelial cells used by Pratt meets the limitation of the present invention. The claims required a cell that is genetically transformed to express an adherence factor. One skilled in the art would immediately realize that, if a cell already expressing an adherence factor were to be genetically altered with a construct that also expresses that factor, the result would necessarily be over-expression of the factor. On the other hand, if the factor that the cell is genetically engineered to express is not one already expressed by a native cell, then the factor would merely be expressed in addition to whatever other adherence factor the cell might express. This is expressly stated in Paragraph [0075] of the Substitute Specification.

While the above fact would be intuitively understood by those skilled in the art, applicant has amended the claims to clarify the point. The amendments are fully supported by the specification as the above discussion points out.

The examiner is requested to reconsider and withdraw the rejection.

**35 USC 102(b) rejection of claims 1, 6, 19, 23 and 51**

The examiner rejected claims 1, 6, 19, 23 and 51 under 102(b) as being anticipated by Huber, et al., *Journal of Vascular Surgery*, 1995, 22(6):795 – 803. The examiner states that Huber discloses a prosthetic vascular ePTFE graft seeded with transduced (genetically transformed) endothelial cells from a vein. The examiner again refers to the statement in the Office Action that the natural expression of an adherence factor meets the claim limitation of a genetic transformation of the cell to express an adherence factor and permits Huber to anticipate the claims. Applicant traverses.

**Applicant's response**

Huber in fact teaches away from the present application. Further, Huber contradicts the examiner's statement that natural expression of an adherence factor meets the limitation of the present application requiring genetic alteration of a cell to express an adherence factor. That is, Huber reports that genetic transformation of endothelial cells results in decreased adherence of the cells in direct contradiction of the teaching of the present application. For example, on page 802, first column, last paragraph, Huber states that:

This control allows us to conclude that the observed decrease in adherence of the transduced ECs was due to the retroviral transduction of the human tPA gene expression.

Then on page 802, second column, first full paragraph, Huber states:

The reported decrease in percent endothelialization may be partly explained by the impaired ability of the tPA-transduced ECs to adhere to the graft surface in vivo and suggests that the tPA retroviral transduction may alter the biologic function of the EC.

One skilled in the art would conclude from the above that genetically transforming ECs would result in poorer adhesion than with native cells and would give no thought to the adherence factors that might or might not be expressed by the wild type cells. Thus, Huber does not anticipate the present invention.

The examiner is requested to reconsider and withdraw the rejection.

#### **Potential 35 USC § 103 rejection of claims**

The examiner notes as an aside that, were applicant to "only amend the claims to read 'overexpress,'" a § 103 rejection could be formulated so as to render the invention unpatentable over Pratt or Huber in view of Nakamura, et al., *J. Biol. Chem.*, **1999**, 274(32):22476 – 83. Applicant disagrees.

#### **Applicant's response**

Nakamura describes at length the DANCE protein and notes that:

In adult vessels, DANCE expression is largely diminished but is reintroduced in balloon-injured vessel and atherosclerotic lesions notably in intimal vascular smooth muscle cells and endothelial cells that lose their ability to proliferate in late state of injury. DANCE protein was shown to promote adhesion of endothelial cells through interaction of integrin and the RGD motif of DANCE.

Neither Pratt nor Huber, however, so much as suggest that genetically transforming endothelial cells to express or over-express a factor such as DANCE

would have any beneficial effect on the adherence of the ECs. Absent the present invention, the consideration of which would constitute impermissible hindsight, there would be no motivation to combine Nakamura with Pratt or Huber to render the present invention obvious.

The examiner is requested to reconsider the possible rejection and abandon it.


### CONCLUSION

In view of the amendments herein and the remarks above, applicant believes that the present application is in condition for allowance and respectfully requests that it be passed to issue.

Applicant hereby petitions for a three month extension in time to respond to the Office Action under 37 CFR 1.136. The Commissioner is authorized to charge the appropriate fee under 37 CFR 1.17 to Bingham McCutchen, LLP Deposit Account No. 50-2518, Docket No. 274,266, Billing Ref.: 24783-7004.

Respectfully submitted,

**BINGHAM MCCUTCHEN LLP**

By:   
Bernard F. Rose, Reg. No. 42,112

Date: January 22, 2003

Three Embarcadero Center, Suite 1800  
San Francisco, CA 94111-4067  
Telephone: (650) 849-4902  
Facsimile: (650) 849-4800

## ADDENDUM A

### VERSION OF CLAIMS SHOWING CHANGES MADE

1. (Amended) An artificial graft comprising a synthetic tubular element having a luminal surface [being] coated with a plurality of endothelial cells [being] that have been genetically transformed to express or over-express at least one endothelial cell proliferating growth factor and at least one cellular adherence factor.
2. (Amended) The artificial vascular graft of claim 1, wherein a first portion of said plurality of endothelial cells is genetically transformed to express or over-express said at least one endothelial proliferating growth factor and [further wherein] a second portion of said plurality of endothelial cells is genetically transformed to express or over-express said at least one cellular adherence factor.
3. (Amended) The artificial vascular graft of claim 1, wherein said luminal surface [is of a substance selected from the group consisting of] comprises polytetrafluoroethylene (PTFE), expanded polytetrafluoroethylene (dPTFE), polyester fiber, poly(ethylene glycol-co-terephthalic acid) (DACRON) [and] or processed animal blood vessels.
4. (Amended) The artificial vascular graft of claim 1, wherein said synthetic tubular element is of an inner [cross sectional] cross-sectional area which is substantially equivalent to an inner [cross sectional] cross-sectional area of a blood vessel.
5. (Amended) The artificial vascular graft of claim 4, wherein said inner [cross sectional] cross-sectional area of said synthetic tubular element is [within a range of] about 7 to about 700 mm<sup>2</sup>.

6. (Amended) The artificial vascular graft of claim 1, wherein said plurality of endothelial cells are [derived] obtained from [a source selected from the group consisting of] a segment of a vein or circulating endothelial cells [,] or are derived from bone marrow progenitor cells[,] or peripheral blood stem cells [and circulating endothelial cells].

7. (Amended) The artificial vascular graft of claim 1, wherein said plurality of endothelial cells are [derived] obtained from [a] an intended recipient of the artificial graft.

8. (Amended) The artificial vascular graft of claim 1, wherein said plurality of endothelial cells are [derived] obtained from a human or animal donor.

9. (Amended) The artificial vascular graft of claim 1, wherein said plurality of endothelial cells form a confluent monolayer at said [inner] luminal surface.

19. (Amended) A method of producing an artificial vascular graft, the method comprising the steps of:

(a) genetically transforming endothelial cells to express or over-express at least one endothelial cell proliferation growth factor and at least one cellular adherence factor; and,

(b) culturing said endothelial cells with a synthetic tubular element having a luminal surface until sufficient endothelialization of said luminal surface is achieved.

20. (Amended) The method of claim 19, wherein step (a) is effected by genetically transforming a first portion of said endothelial cells to express or over-express said at least one endothelial proliferation growth factor and genetically transforming a second portion of said endothelial cells to express or over-express said at least one cellular adherence factor.

23. (Amended) The method of claim 19, wherein said endothelial cells are [derived] obtained from [a source selected from the group consisting of] a segment of a vein or circulating endothelial cells[, and] or are derived from bone marrow progenitor cells[,] or peripheral blood stem cells[ and circulating endothelial cells].

51. (Amended) A method of producing genetically transformed endothelial cells, the method comprising the steps of:

(a) obtaining endothelial cells from a source selected from the group consisting of a segment of a vein and bone marrow progenitor cells; and,

(b) transforming said endothelial cells to express or over-express at least one endothelial cell proliferation growth factor and at least one cellular adherence factor concurrently.

52. (Amended) A method of replacing or bypassing at least a portion of a vascular system of an individual, the method comprising the step of implanting into the vascular system of the individual an artificial vascular graft[,], so as to form a fluid communication between the vascular system and said artificial vascular graft, said artificial vascular graft [including] comprising a synthetic tubular element having a luminal surface [being] coated with a plurality of endothelial cells [being] genetically transformed to express or over-express at least one endothelial cell proliferating growth factor and at least one cellular adherence factor.

53. (Amended) The method of claim 52, wherein a first portion of said plurality of endothelial cells is genetically transformed to express or over-express said at least one endothelial cell proliferation growth factor and [further wherein] a second portion of said plurality of endothelial cells is genetically transformed to express or over-express said at least one cellular adherence factor.



54. (Amended) The [artificial vascular graft] method of claim 52, wherein said synthetic tubular element [is of] has an inner [cross sectional] cross-sectional area which is substantially equivalent to an inner [cross sectional] cross-sectional area of a blood vessel.

55. (Amended) The [artificial vascular graft] method of claim 54, wherein said inner [cross sectional] cross-sectional area of said synthetic tubular element is [with a range of] about 7 to about 700 mm<sup>2</sup>.



**274/266**  
**24783-7004**

**SUBSTITUTE  
SPECIFICATION**

**MARKED-UP VERSION**

**ARTIFICIAL VASCULAR GRAFTS, AND METHODS OF  
PRODUCING AND USING SAME**

**[FIELD AND BACKGROUND OF THE INVENTION]**

**FIELD OF THE INVENTION**

[0001] The present invention relates to artificial vascular grafts. More particularly, the present invention relates to genetically transformed cells and methods of generating same, which cells are useful in the generation of improved artificial vascular grafts. The present invention further relates to methods of using the artificial vascular grafts for therapy of vascular diseases.

**BACKGROUND OF THE INVENTION**

***[Vascular diseases:]***

[0002] Vascular diseases are a major cause of morbidity and mortality in the developed world and thus affects a large part of the population. Bypass surgery utilizing venous, arterial or synthetic conduit grafts is often used to alleviate the clinical sequelae of these diseases by allowing or restoring blood flow around a blocked or damaged blood vessel. It is estimated that over 1 million such procedures are performed in developed countries annually and that 30-50% of these interventions fail within 5 to 7 years (1-4).

***[Arterial grafts:]***

[0003] Arterial grafts have a higher durability rate in the long term when compared to venous and synthetic grafts (5). Yet, arterial grafts can be used only in limited clinical circumstances such as, for example, aorto-coronary bypass surgery. There is an ever-increasing need for synthetic grafts with a biological-compatible surface of high durability over extended periods of time. The demand for small caliber grafts is especially high in lesions below the

femoral artery and in patients who have limited native vessels such as with varicose veins or previous bypass surgery (2,3). Use of polytetrafluoroethylene (PTFE) and other materials in synthetic grafts has gained popularity due to the biocompatibility of the material itself and to its durability. Modification of the synthetic material was described in many animal studies and in a few human studies (5-10).

[0004] To improve function and durability of synthetic grafts, autologous endothelial cells have been used to seed grafts before implantation. Endothelial cells provide the best bio-compatible surface, and may provide long term protection from thrombosis due to their thrombolytic capacity (11,12). In addition, endothelial cell coverage can also prevent neointimal proliferation and inflammatory reaction in the graft (13). However, to date, randomized large scale studies have not proven that endothelial cell seeding can improve graft durability (8-10).

[0005] Endothelial cells used for seeding grafts before implantation are typically harvested from short venous segments or from adipose tissue. Other sources may be cells from transgenic animals having human characteristics or autologous endothelial cells from the individual patient. Another possible source for endothelial cells is progenitor cells isolated from the peripheral blood or bone marrow.

[0006] New strategies have been attempted at the stage of artificial graft design and preparation in order to improve efficiency of artificial vascular grafts utilized in treating human vascular diseases. Complete endothelialization of artificial vascular graft luminal surface is still extremely delayed and has a major impact on graft patency. Therefore, a major task is to improve both graft coverage and cell adherence to the graft matrix. Previous attempts in which endothelial cells were seeded onto synthetic grafts, resulted in incomplete endothelialization as well as cell detachment upon exposure to blood flow conditions.

[0007] One of the most recent techniques of improving graft endothelialization is to coat it with genetically transformed cells having better proliferating capacity.

[0008] To enhance differentiation of progenitor bone marrow cells and provide a short and reliable method for generating endothelial cells useable in artificial graft preparation, such cells are typically transformed with a vascular endothelial growth factor (VEGF) expression vector which when expressed in the cell, can enhance the efficiency of endothelial cell isolation and growth. Thus, the use of a specific endothelial cell mitogen such as VEGF, can shorten the time period from cell harvesting to seeding enabling lower initial graft seeding densities to be used.

[0009] Endothelial cell proliferation and migration is initiated by activation of VEGF receptors (16). Although three VEGF receptors have been identified and characterized, it is the *KDR* receptor that plays a key role in the angiogenic switch that is comprised of proliferation and migration (16,17).

[0010] VEGF offers distinct advantages over other less endothelial cell-specific growth factors that might enhance graft endothelialization since it has reduced impact on other vascular cells, in particular smooth muscle cells, and as such reduces the potential for adverse stimulatory effects. Thus, VEGF released by genetically modified cells seeded onto artificial graft will recruit endothelial cells, but not smooth muscle cells, from anastomosis sites.

[0011] Although genetically transfected endothelial cells over-expressing VEGF are capable of enhanced growth as compared to non-transformed endothelial cells, artificial vascular grafts incorporating such transformed cells are functionally limited since the transformed cells coating the luminal surface of the graft are incapable of withstanding flow forces following implantation.

[0012] There is thus a widely recognized need for and it would be highly advantageous to have an artificial vascular graft capable of supporting blood flow following implantation and yet devoid of the abovementioned limitations of prior art grafts.

#### **[SUMMARY OF THE INVENTION]**

### **SUMMARY OF THE INVENTION**

[0013] According to one aspect of the present invention there is provided an artificial vascular graft comprising a synthetic tubular element having a luminal surface being coated with a plurality of endothelial cells comprising the steps of: (a) genetically transforming endothelial cells to express at least one endothelial cell proliferating growth factor and at least one cellular adherence factor; and (b) seeding and culturing the endothelial cells within a synthetic tubular element having a luminal surface until sufficient endothelialization of the luminal surface is achieved.

[0014] According to further features in preferred embodiments of the invention described below, a first portion of the plurality of endothelial cells is genetically transformed to express the at least one endothelial cell proliferating growth factor and further wherein a second portion of the plurality of endothelial cells is genetically transformed to express the at least one cellular adherence factor.

[0015] According to still further features in the described preferred embodiments the luminal surface is of a substance selected from the group consisting of polytetrafluoroethylene (PTFE), expanded polytetrafluoroethylene (ePTFE), polyester fibers, Dacron and processed animal blood vessels. According to still further features in the described preferred embodiments the synthetic tubular element is of an s inner cross sectional area which is substantially equivalent to an inner cross sectional area of a blood vessel.

[0016] According to still further features in the described preferred embodiments the inner cross sectional area of the synthetic tubular element is within a range of about 7 to 700 mm<sup>2</sup>. According to still further features in the described preferred embodiments the plurality of endothelial cells are derived from a source selected from the group consisting of a segment of a vein, bone marrow, peripheral blood progenitor cells and circulating endothelial cells.

[0017] According to still further features in the described preferred embodiments the plurality of endothelial cells are derived from the recipient of the artificial vascular graft.

- [0018] According to still further features in the described preferred embodiments the plurality of endothelial cells are derived from embryonic or mature tissues of a human or an animal donor.
- [0019] According to still further features in the described preferred embodiments the plurality of endothelial cells form a confluent monolayer at the inner luminal surface.
- [0020] According to still further features in the described preferred embodiments at least one endothelial cell proliferating growth factor is selected from the group consisting of VEGF, basic or acidic FGF, HGF and any other endothelial growth factor.
- [0021] According to still further features in the described preferred embodiments at least one cellular adherence factor is DANCE, UP50 or any other molecule capable of promoting cellular adherence to graft material.
- [0022] According to still further features in the described preferred embodiments the plurality of endothelial cells are further genetically transformed to express at least one marker polypeptide.
- [0023] According to still another aspect of the present invention there is provided a nucleic acid expression construct comprising: (a) a first polynucleotide segment encoding an endothelial cell proliferating growth factor; and (b) a second polynucleotide segment encoding cellular adherence factor.
- [0024] According to still another aspect of the present invention there is provided a nucleic acid expression construct system comprising: (a) a first nucleic acid expression construct including a first polynucleotide segment encoding an endothelial cell proliferating growth factor; and (b) a second nucleic acid expression construct including a second polynucleotide segment encoding cellular adherence factor.
- [0025] According to still further features in the described preferred embodiments the first and the second nucleic acid expression constructs further include at least one additional polynucleotide segment encoding a marker polypeptide.
- [0026] According to still another aspect of the present invention there is provided a kit comprising a stand for engaging at least one tube, the at least

one tube including a nucleic acid expression constructs system, the nucleic acid expression construct system including: (a) a first nucleic acid expression construct including a first polynucleotide encoding an endothelial cell proliferating growth factor; and (b) a second nucleic acid expression construct including a second polynucleotide encoding cellular adherence factor.

[0027] According to still another aspect of the present invention there is provided a kit comprising a stand for engaging at least one tube, the at least one tube including a nucleic acid expression construct, the nucleic acid expression construct including: (a) a first polynucleotide encoding an endothelial cell proliferating growth factor; and (b) a second polynucleotide encoding a cellular adherence factor.

[0028] According to still another aspect of the present invention there is provided a method of producing genetically transformed endothelial cells, the method comprising the steps of: (a) obtaining endothelial cells from a source selected from the group consisting of a segment of a vein, bone marrow progenitor cells, peripheral blood stem cells and circulating endothelial cells; and (b) transforming the endothelial cells to express at least one endothelial cell proliferating growth factor and at least one cellular adherence factor concurrently or separately.

[0029] According to still further features in the described preferred embodiments, the nucleic acid expression construct further comprising at least one promoter sequence being for directing the expression of at least one of the first and the second polynucleotide segments.

[0030] According to still further features in the described preferred embodiments, the first polynucleotide segment is transcriptionally linked to the second polynucleotide segment whereas the first and the second polynucleotide segment are under the transcriptional control of a single promoter sequence of the at least one promoter sequence.

[0031] According to still further features in the described preferred embodiments the nucleic acid construct further comprises a linker sequence being interposed between the first and the second polynucleotide segments.



[0032] According to still further features in the described preferred embodiments, the linker sequence is selected from the group consisting of IRES and a protease cleavage recognition site.

[0033] According to still further features in the described preferred embodiments, at least one promoter is functional in eukaryotic cells.

[0034] According to still further features in the described preferred embodiments, at least one promoter is selected from the group consisting of a constitutive promoter, an inducible promoter and a tissue specific promoter.

[0035] According to still further features in the described preferred embodiments, the nucleic acid expression construct further comprises: (c) a first promoter sequence being for directing the expression of the first polynucleotide segment; and (d) a second promoter sequence being for directing the expression of the second polynucleotide segment.

[0036] According to still further features in the described preferred embodiments, the first promoter and the second promoter are selected from the group consisting of a constitutive promoter, an inducible promoter and a tissue specific promoter.

[0037] According to still further features in the described preferred embodiments, the inducible promoters are regulatable by same effector molecule.

[0038] According to still further features in the described preferred embodiments, the nucleic acid expression construct further comprises at least one additional polynucleotide segment encoding a marker polypeptide.

[0039] According to still further features in the described preferred embodiments, the marker polypeptide is selected from the group consisting of a selection polypeptide and a reporter polypeptide.

[0040] According to still further features in the described preferred embodiments, at least one additional polynucleotide segment is transcriptionally linked to the at least one of the first and the second polynucleotide segments.

[0041] According to still further features in the described preferred embodiments, at least one additional polynucleotide segment is

transcriptionally linked to the at least one of the first and the second polynucleotide segments via linker segment.

[0042] According to still further features in the described preferred embodiments, the linker sequence is selected from the group consisting of IRES and a protease cleavage recognition site.

[0043] According to still further features in the described preferred embodiments, at least one additional polynucleotide segment is translationally fused to at least one of the first and the second polynucleotide segments.

[0044] According to still another aspect of the present invention, there is provided a transformed endothelial cell being genetically transformed to express at least one endothelial cell proliferating growth factor and at least one cellular adherence factor.

[0045] According to still further features in the described preferred embodiments, the transformed endothelial cell further genetically transformed to express at least one marker polypeptide.

[0046] According to still further features in the described preferred embodiments, the endothelial cell is derived from a source selected from the group consisting of a segment of a vein, bone marrow, peripheral blood progenitor cells and circulating endothelial cells.

[0047] According to still further features in the described preferred embodiments, the endothelial cell is derived from a source selected from the group consisting of embryonic or mature tissues of a human donor and an animal source.

[0048] According to still further features in the described preferred embodiments, the cellular proliferating growth factor is selected from the group consisting of VEGF, acidic and basic FGF, HGF and any other endothelial cell growth factor.

[0049] According to still further features in the described preferred embodiments the cellular adherence factor is DANCE, UP50 or any other molecule capable of promoting cellular adherence to graft material.

[0050] The present invention successfully addresses the shortcomings of the presently known configurations by providing an artificial vascular graft

and method of generating same, which graft is useful for vascular replacement or vascular bypass.

## **DETAILED DESCRIPTION OF THE INVENTION**

### **Brief description of the drawings**

[0051] The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice

[0052] [In the drawings:]

[0053] FIG. 1 is a perspective view of an artificial vascular graft of the present invention.

[0054] FIGs. 2a-c illustrate VEGF expression vector constructs according to the teachings of the present invention.

[0055] FIGs. 3a-c illustrates a Urine p50 protein (UP50) or a neural crest epidermal growth factor-like (DANCE) expression vector construct according to the teachings of the present invention.

[0056] [FIG. 4a illustrates a PTFE (polytetrafluoroethylene) graft seeded with human endothelial cells. The cells were infected with recombinant adenoviral vector encoding nuclear targeted LacZ gene. Blue staining indicates an infected cell expressing the transgene. Staining was performed 24 hours following infection.

[0057] FIG. 4b illustrates primary human endothelial cells seeded on a PTFE graft and infected with recombinant adenoviral vector co-expressing the

VEGF and green fluorescent protein (GFP) genes. Green cells co-express VEGF and GFP.

[0058] FIG. 5a illustrates a PTFE graft seeded with primary human and sheep venous endothelial cells following transformation with recombinant pseudo-typed retroviral vector encoding the LacZ gene. Blue staining indicates an infected cell expressing the transgene.

[0059] FIG. 5b illustrates primary sheep endothelial cells seeded on a PTFE graft following transformation with recombinant pseudo-typed retroviral vector co-expressing VEGF and GFP. Green cells indicate cells co-expressing VEGF and GFP.

[0060] FIGs. 6a-b illustrate binding and cross linking of  $^{125}\text{I}$ -VEGF to specific receptors expressed on human saphenous vein endothelial cells (HSVEC). VEGF receptor expression in EC was detected at day 2 (Figure 6a) and day 5 (Figure 6b) following adenoviral infection. Cells were washed extensively and incubated with  $^{125}\text{I}$ -VEGF (1 ng/ml or 5 ng/ml). Binding specificity was detected by competition in the presence of 1.5  $\mu\text{g/ml}$  unlabeled recombinant VEGF<sub>165</sub> (marked as + lanes at the top of Figure 6a). The cells were lysed and  $^{125}\text{I}$ -VEGF -receptor complexes were separated on 6% polyacrylamide gel and exposed to XAR film for autoradiography. The lanes in Figures 6a-b are as follows: 1. control uninfected cells; 2. cells infected with rAdlacZ; 3. cells infected with rAdVEGF; 4. uninfected EC supplemented with recombinant VEGF<sub>165</sub> (2 ng/ml); 5. uninfected EC supplemented with recombinant VEGF<sub>165</sub> (10 ng/ml); and 6. uninfected EC supplemented with conditioned medium collected from rAdVEGF infected cells.]

## **[DESCRIPTION OF THE PREFERRED EMBODIMENTS]**

### **Discussion**

[0061] The present invention is of an artificial vascular graft and methods of generating same which can be utilized to replace or bypass damaged and/or occluded blood vessels. Specifically, the present invention is of a method of generating synthetic vascular grafts coated with mammalian

endothelial cells which grafts can provide a viable alternative to presently utilized natural and synthetic vascular grafts.

[0062] The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

[0063] Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

[0064] Artificial grafts are yet to be extensively utilized in surgical bypass procedures. One of the limitations plaguing presently available artificial vascular grafts as well as some natural vascular grafts is the lack of durability over extended periods of time. Although the use of endothelial cells greatly enhances the quality of coated artificial grafts, by improving performance in general and by improving thrombolytic activity in particular, such grafts typically suffer from loss of cell coating under blood flow forces since the coated cells cannot withstand the forces exerted thereupon by the flow of blood.

[0065] Thus, the present inventors have suggested that a genetically modified population of endothelial cells which co-express a cellular adherence factor, such as DANCE or UP50 along with a cellular growth factor, such as VEGF, or a mixture of two subpopulations of genetically modified endothelial cells, one expressing a cellular adherence factor and the other an endothelial cell growth factor are advantageous for use in artificial vascular grafts since the presence of a cellular adherence factor in these cells greatly increases the adherence to the luminal surface of the artificial graft substrate which greatly increases the durability of these vascular grafts. The proteins expressed and secreted by such cells will lead to the complete coverage of the grafts with

endothelial cells which are capable of withstanding the forces exerted by the flow of blood therein.

[0066] Thus according to one aspect of the present invention and as specifically shown in Figure 1, there is provided an artificial vascular graft which is referred to herein as graft **10**.

[0067] Graft **10** includes a synthetic tubular element **12** having a luminal surface **14**. Preferably, synthetic tubular element **12** is of an inner cross sectional area of about 7 to 700 mm<sup>2</sup> or any cross sectional area or diameter which is substantially equivalent to an inner cross sectional area or diameter of a blood vessel.

[0068] Luminal surface **14** is fabricated from a substance including, but not limited to, polytetrafluoroethylene (PTFE), expanded polytetrafluoroethylene (ePTFE), polyester fibers, Dacron™ or processed blood vessels derived from an animal or a human.

[0069] As is further described hereinunder and in the Examples section which follows, luminal surface **14** is coated with a plurality of endothelial cells **16** which are genetically transformed to express at least one endothelial cell proliferating growth factor and at least one cellular adherence factor. Luminal surface **14** preferably includes structures such as pits or projection which facilitate adherence of endothelial cells **16** to surface **14**.

[0070] Graft **10** is fabricated by genetically transforming a population of endothelial cells **16** with at least one endothelial cell proliferating growth factor and at least one cellular adherence factor and culturing the transformed cells within element **12** until sufficient endothelialization of luminal surface **14** is achieved.

[0071] It will be appreciated that the population of endothelial cells **16** can be transformed with both the endothelial cell proliferating growth factor and the cellular adherence factor, or alternatively a first portion of such population can be transformed with the endothelial cell proliferating growth factor, while a second portion of this population is transformed with cellular adherence factor.

[0072] As is further described in the examples section which follows endothelial cells **16** are cultured with element **12** in a manner which is conducive for the formation of a confluent monolayer upon luminal surface **14**.

[0073] According to a preferred embodiment of the present invention, endothelial cells **16** are prepared from, for example, a segment of a vein, bone marrow progenitor cells, peripheral blood stem cells or circulating endothelial cells of an intended recipient of graft **10** or a syngeneic donor.

[0074] Alternatively xenogeneic tissue can also be utilized for the separation of endothelial cells **16** providing measures are taken prior to, following fabrication of graft **10**, so as to avoid cell rejection. Numerous methods for preventing or alleviating rejection are known in the field and as such no further detail is given herein.

[0075] As is further described in the Examples section below, the co-expression of a cellular adherence factor along with a cellular growth factor enables endothelial cells **16** of graft **10** of the present invention to withstand flow forces which would normally lead to endothelial cell loss from the luminal surface of prior art artificial vascular grafts.

[0076] Thus, graft **10** of the present invention can be successfully utilized in vascular surgical procedures aimed at replacing or bypassing a damaged or occluded blood vessel.

[0077] Thus, according to another aspect of the present invention there is provided a method of replacing or bypassing at least a portion of a vascular system of an individual. The method is effected by implanting into the vascular system of the individual graft **10**, so as to form a fluid communication between the vascular system and graft **10**.

[0078] As used herein the phrase "genetic transformation" refers to the introduction of exogenous polynucleotide sequences into a cell. Such sequences can integrate into the genome of the cell, or alternatively, such exogenous sequences can exist in the nucleus or cytoplasm of the cell in a transient manner.

[0079] The transformed endothelial cells according to the teachings of the present invention express at least one endothelial cell proliferating growth factor and at least one cellular adherence factor. Such factors can be

expressed from exogenous polynucleotide sequences introduced into the endothelial cells, or alternatively such factors can be endogenous factors naturally expressed by the cell in which case, the cell is transformed with sequences directed at causing the over expression of the endogenous factors.

[0080] As used herein, the term “over-expression” refers to expression levels which exceeded those normally found in a cell. Over expression can be effected by introducing additional copies of an endogenous gene into a cell thus increasing the copy number of the endogenous gene or genes expressed in a cell, or by introducing enhancer sequence elements via gene knock-in procedures, which elements up-regulate the transcription or translation of the endogenous genes.

[0081] The exogenous polynucleotide sequences are preferably included in one or more nucleic acid constructs which are used to transform endothelial cells.

[0082] Thus according to another aspect of the present invention there is provided a nucleic acid expression construct useful for generating endothelial cells 16 of the present invention. The construct includes a first polynucleotide segment encoding an endothelial cell proliferating growth factor and a second polynucleotide segment encoding a cellular adherence factor.

[0083] According to preferred embodiments of the present invention, the endothelial cell proliferating growth factor is, for example, VEGF, acidic or basic FGF or HGF, while the cellular adherence factor is, for example, DANCE, UP50 or any other molecule capable of promoting endothelial cell adherence to the graft material. Such factors can be encoded by sequences derived from human cells or any other mammalian cells provided the expressed factors are functional in endothelial cells.

[0084] The construct according to this aspect of the present invention also includes one or more promoter sequences for directing the expression of the first and second polynucleotide segments in endothelial cells. Such a promoter can be any mammalian functional promoter which is either constitutive, tissue specific or inducible.



[0085] As is further described in the examples section herein below, the endothelial cell proliferating growth factor and the cellular adherence factor are preferably expressed in a different temporal pattern.

[0086] As such, the construct includes two promoter sequences each s being for directing the expression of a single polynucleotide segment. Preferably such promoters are inducible promoters regulatable by the same effector molecule. Preferably, the promoter sequences are selected such that one promoter is up-regulatable by an effector molecule, while the second promoter is down-regulatable by the same effector.

[0087] Examples of suitable inducible promoters and effectors are given in the Examples section herein.

[0088] Notwithstanding the above, it will be appreciated that a single promoter sequence can also be utilized by the construct of the present invention provided the first and second polynucleotide segments are transcriptionally linked and provided that an internal ribosome entry site (IRES) is included for directing the translation of the second segment of the polycistronic message transcribed.

[0089] The two polynucleotide segments of the construct according to this aspect of the present invention can also be translationally fused provided a protease cleavage site is provided between them to allow cleavage and separation of the two polypeptides in endothelial cells.

[0090] It will further be appreciated that each of the two polynucleotide segments can be included in a separate nucleic acid constructs, which can be co-introduced into endothelial cells.

[0091] Thus, according to yet another aspect of the present invention there is provided a nucleic acid expression construct system useful in generating endothelial cells 16 of the present invention. The construct system of this aspect of the present invention includes a first nucleic acid expression construct including a first polynucleotide segment encoding an endothelial cell proliferating growth factor and a second nucleic acid expression construct including a second polynucleotide segment encoding a cellular adherence factor.

[0092] The construct or construct system of the present invention preferably also includes additional polynucleotide segments encoding marker genes, selection marker genes and the like, so as to enable selection of transformed cells and/or monitoring of the expression of the endothelial cell proliferating growth factor and/or the cellular adherence factor. Examples of suitable reporter genes include, but are not limited to, beta-galactosidase, luciferase, GFP and the like. Examples of selection markers include antibiotic resistance genes and the like.

[0093] It will be appreciated that to monitor expression of the endothelial cell proliferating growth factor and/or the cellular adherence factor, the reporter gene can be provided in transcriptional or translational fusion to the polynucleotide segment encoding the factor or it can be placed under a transcriptional control of a promoter sequence identical to that directing the transcription of the factor.

[0094] It will be appreciated that the polynucleotide segments encoding the endothelial cell proliferating growth factor and/or the cellular adherence factor, can be ligated into a commercially available expression vector system suitable for transforming mammalian cells and for directing the expression of these factors within the transformed cells. It will be appreciated that such commercially available vector systems can easily be modified via commonly used recombinant techniques in order to replace, duplicate or mutate existing promoter or enhancer sequences and/or introduce any additional polynucleotide sequences.

[0095] Suitable mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.I, which are available from Invitrogen, pCI which is available from Promega, pBK-RSV and pBK-CMV which are available from Stratagene, pTRES which is available from Clontech, and their derivatives.

[0096] As mentioned above, the endothelial cells of the present invention can also include exogenous polynucleotide sequences directed at over-expressing endogenously encoded factors.

[0097] Thus, according to yet another aspect of the present invention, there is provided a nucleic acid construct or construct system which serves to up-regulate expression of endogenously encoded factors. Such a construct or constructs can include transcriptional regulatory sequences which when provided in cis to endogenous sequences encoding the endothelial cell proliferating growth factor and/or the cellular adherence factor, can up-regulate the transcription thereof.

[0098] Alternatively, such a construct or constructs can encode translational regulatory sequences which when provided in trans to endogenous sequences encoding the endothelial cell proliferating growth factor and/or the cellular adherence factor, can up-regulate the translation thereof.

[0099] Standard gene knock-in techniques can be utilized to introduce cis acting transcriptional regulatory sequences into a genome of the endothelial cells. For a review of gene knock-in methodology see, for example, United States Patent Nos. 5,487,992, 5,464,764, 5,387,742, 5,360,735, 5,347,075, 5,298,422, 5,288,846, 5,221,778, 5,175,385, 5,175,384, 5,175,383, 4,736,866 as well as Burke and Olson, *Methods in Enzymology*, 194:251-270, 1991; Capecchi, *Science* 244:1288-1292, 1989; Davies *et al.*, *Nucleic Acids Research*, 20 (11) 2693-2698, 1992; Dickinson *et al.*, *Human Molecular Genetics*, 2(8):1299-1302, 1993; Duff and Lincoln, "Insertion of a pathogenic mutation into a yeast artificial chromosome containing the human APP gene add expression in ES cells", *Research Advances in Alzheimer's Disease and Related Disorders*, 1995; Huxley *et al.*, *Genomics*, 9:742-750 1991; Jakobovits *et al.*, *Nature*, 362:255-261 1993; Lamb *et al.*, *Nature Genetics*, 5: 22-29, 1993; Pearson and Choi, *Proc. Natl. Acad. Sci. USA*, 1993, 90:10578-82; Rothstein, *Methods in Enzymology*, 194:281-301, 1991; Schedl *et al.*, *Nature*, 362: 258-261, 1993; Strauss *et al.*, *Science*, 259:1904-1907, 1993, WO 94/23049, WO 93/14200, WO 94/06908 and WO 94/28123 also provide information.

[00100] The nucleic acid constructs of the present invention can be introduced into endothelial cells via any standard mammalian transformation method. Such methods include, but are not limited to, direct DNA uptake

techniques, and virus or liposome mediated transformation (for further detail see, for example, "Methods in Enzymology" Vol. 1-317, Academic Press).

[0100] Thus, the present invention provides an improved artificial vascular graft which is internally coated with genetically transformed endothelial cells expressing a cellular growth factor and a cellular adhesion factor. Expression of the cellular adherence factor, in addition to the cellular growth factor, enables the endothelial cells of the coating to adhere with greater force to the luminal surface of the artificial vascular graft thereby providing a durable cellular coating which can withstand, for extended periods of time, the flow forces exerted upon the luminal surface of the artificial vascular graft following implantation.

[0101] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

#### **EXAMPLES**

[0102] Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non-limiting fashion.

[0103] Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-

III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein.] Other general references are provided throughout this document. The procedures therein are believed to be well known in the art [and] so the references are provided solely for the convenience of the reader. [All the information contained therein is incorporated herein by reference.]

#### **EXAMPLE 1**

[0104] Since artificial graft coating with endothelial cells is of a great importance in surgical treatment of vascular diseases, a system was developed which enables complete endothelialization of artificial grafts expressing at least one endothelial cell proliferating growth factor and at least one cellular adherence factor. To generate such grafts a population of endothelial cells utilized as graft coating are transformed with several expression vectors as describe below.

**Expression vectors and virus particles: Construction of rAdGFP and rAdVEGF-GFP recombinant Adenoviral-based vectors.**

[0105] The recombinant adenoviral vectors expressing the human VEGF165 and/or the GFP genes were constructed in two steps. The first step included construction of two shuttle vector plasmids expressing the GFP and the VEGF165 -GFP genes. For this purpose a shuttle vector plasmid containing the left arm (16%) of the Ad5 genome (A kind gift from Shenk T, Princeton University, NJ, USA) with a deletion in the EI region was used. This deletion was replaced by IRES-GFP fragment (Clontech, Ca, USA) regulated by the CMV5 promoter. This shuttle plasmid was used for construction of rAdGFP. A second shuttle vector was constructed by ligating a 600bp BamHI fragment containing the human VEGF165 cDNA which includes the secretion signal sequence (Genbank Accession number AB021221) into the BglII site between the CMV5 promoter and the IRES sequence. This bi-cistronic shuttle vector which expresses both the VEGF165 and the GFP genes was used for construction of rAdVEGF-GFP.

[0106] The second step was effected via homologous recombination between each shuttle vector and the adenoviral plasmid (pJM17 a kind gift from Shenk T, Princeton University, NJ, USA) via co-transfection into the 293 cell line. The pJM17 plasmid contained the adenovirus genome, excluding the E3 region, and an insert (pBRX) in the EI region of the virus.

[0107] Homologous recombination between the expression plasmid and pJM17 following transfection into the 293 cells replaced the EI region and pBRX insert with the expression vector from the shuttle plasmid. Plaque formation occurred between 2 to 4 weeks following co-transfection. Individual plaques were isolated and viral extracts were amplified by infecting a 293 cell line. Titer of viral stocks, as determined by plaque assay in these cells, ranged from  $10^{10}$  to  $10^{11}$  pfu/ml. Expression of VEGF was confirmed by western blotting while expression of GFP was confirmed using an inverted fluorescent microscope. Recombinant vectors encoding the nuclear targeted beta-galactosidase (LacZ) (Clontech, CA, USA) were prepared using similar methods.

**Preparation of retro virus vector expressing the VEGF165 -GFP genes**

[0108] Figures 2a-c illustrate the VEGF-GFP expression vector constructs according to the teachings of the present invention. The recombinant retroviral vectors expressing the human VEGF165 and/or the EGFP genes (EGFP -Clontech, Ca, USA) were constructed by separately cloning two different vectors into the LXSN plasmid (Clontech, Ca, USA). For construction of LXSN-GFP expressing EGFP alone, an EcoRI-HpaI 1400bp fragment containing the IRES element and EGFP gene was inserted into the specific restriction sites of the LXSN plasmid multiple cloning site (MCS). For construction of the LXSN-VEGF-GFP bi-cistronic plasmid which co-expresses the VEGF165 and the EGFP genes, a 2.0 kB EcoRJ-MunI fragment containing VEGF165 (600bp), and the IRES and EGFP sequences were ligated into the EcoRI restriction site of the LXSN MCS. Expression from both vectors is regulated by the MoMULV long terminal repeat (LTR) (Clontech, Ca, USA). For retroviral vectors production, 10 µg of LXSN-GFP or LXSN-VEGF-GFP plasmid DNA were transfected into 293E3 ecotopic packaging cells and incubated for 48 hours, following which the supernatant from confluent cultures of G418 resistant producer cells were collected, filtered (0.45 µm) and added to PA317 amphotropic packaging cells. The transduced cells were exposed to G418 selection and the supernatant was collected again after 48 hours and used to infect TEFLYGA amphotropic packaging cells which uniquely express the GALV envelope for high transduction efficiency of endothelial cells. This packaging cell line has the additional advantage of producing amphotropic particles resistant to human serum inactivation. Following G418 selection of transduced TEFLYGA cells, individual colonies were collected and screened for EGFP and VEGF165 expression. Viral titers of each colony were determined by TE671 cells transduction. Titters ranged between  $10^5$  and  $10^6$  pfu/ml. The highest-titer producing colonies were selected and their supernatants were collected and stored at -80°C.

[0109] The above described steps can also be applied to the construction of a cellular adherence factor expression vector. For example, a PCR amplified fragment of human derived UP50 nucleotide sequence (Genbank

Accession number AF093118) or DANCE nucleotide sequence (GenBank Accession Number AF112152) can be ligated into a mammalian expression vector suitable for expression of the cellular adherence factor in endothelial cells (Figures 3a-b).

### **Transformed cell preparation**

#### **Autologous endothelial cell harvesting**

[0110] Endothelial cells are harvested from 5cm long venous segments of hind limb. Cells are harvested by collagenase digestion, which is well-known in the art and will not be further described other than to note that [as previously described (Flugelman MY, Virmani R, Leon MB, Bowman RL, Dichek DA. Circulation Research 1992;70:348-354).] cells from passages 3-9 are collected to ensure stability of cell characteristics. Cell identity is tested with immunohistochemistry for von Willebrand's factor.

#### **Bone marrow progenitor cell infection with VEGF encoding vectors**

[0111] Bone marrow, progenitor cells were infected with recombinant adenoviral vectors encoding VEGF-GFP using standard techniques well known in the art. Differentiation is tested in infected cells using morphology and immunohistochemistry for von Willebrand's factor. [For more details of cell harvesting see: Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner JM. EMBO J 1999;18:3964-72, and Huang XL, Takakura N, Suda T. Biochem Biophys Res Commun 1999;264:133-8.]

#### **Regulation of gene expression after seeding**

[0112] An effector regulatable expression system can be used in order to regulate gene expression of VEGF and DANCE in endothelial cells. For example, VEGF expression can be downregulated via tetracycline, while DANCE expression is upregulated thereby. With these systems, the seeded cells can be made to express VEGF in the first week when cell proliferation is needed, while use of tetracycline one week following surgery downregulates VEGF expression and upregulates DANCE expression to increase cell adherence to the grafts. (Agha-Mohammadi S, Lotze MT. Regulatable systems: applications in gene therapy and replicating viruses. J Clinical Investigations 2000; 105:1177-1183.)



**Transduction of human and sheep saphenous vein endothelial cells by retro viral vectors**

[0113] Endothelial cells were seeded (105 cells/35 mm dish) in fibronectin coated plates and grown in M199 containing 20% fetal serum. After 24 hours the cells were exposed to the cationic polymer DEAE-dextran (0.1µg/ml) for 1 hour before transduction, washed 3 times with PBS and then transduced with high titer virus. Following incubation for 4 hours at 37 °C, the virus is replaced by fresh medium containing 20% fetal serum. A day later the cells are trypsinized and seeded again on fibronectin coated PTFE (approximately 1 cm<sup>2</sup>). [Figure 4a-6b illustrate the expression levels from the various constructs as indicated by LacZ or GFP activity.]

**Analysis of KDR binding expression and activation**

[0114] Iodination of 5µg of human recombinant VEGF165 is performed by the chloramine-T method. Free iodine is separated from 125I-VEGF165 using heparin-Sepharose affinity chromatography. The specific activity of 125I-VEGF165 obtained is usually 0.5-1.5 x 10<sup>5</sup> cpm/ng. For more details see: Vaisman N, Gospodarowicz D, Neufeld G. J Biol Chem 1990;265:19461-19466.

[0115] The grafts are cut longitudinally and placed in a cell culture dish and then washed in cold PBS. The binding of 125I-VEGF165 to the cell surface receptors is carried out in binding buffer for 2 hours at 4 °C followed by cross linking in the presence of disuccinimidyl substrate. Cells seeded on the grafts are lysed by lysis buffer and the VEGF-receptor complexes are separated on SDS-polyacrylamide gel and analyzed by autoradiography. The gels are stained by Coomassie-blue to assess the amounts of loaded proteins. Immunoprecipitation for the phosphorylated and unphosphorylated fractions of KDR is used for the evaluation of KDR activation. Results of the binding assay are shown in Figures 6a-b. Table 1 represents the KDR and the neuropilins bands intensity at day 5 following infection (5 ng/ml <sup>125</sup>I-VEGF treated cells). Numbers represent relative densitometric values following standardization of the protein quantity loaded on the gel.

**Table 1**

Lanes:	1	2	3	4	5	6
bands:	Control	rAdLacZ	rAdVEGF	VEGF <sub>165</sub> 2 ng/ml	VEGF <sub>165</sub> 10 ng/ml	rAdVEGF Supernatant
KDR:	1	0.85	6	0.9	7.2	6.4
Neuropilins:	1	0.77	5.2	1.3	2.5	4.3

**Analysis of recombinant protein expression**

[0116] VEGF is measured in supernatant collected from tissue culture dishes containing the seeded grafts. ELISA is used to measure VEGF production over 24 hours period. Similar methods will be used for the DANCE protein.

**EXAMPLE 2*****[Synthetic Grafts seeding techniques]***

[0117] Grafts seeding techniques are detailed in: Tseng, D.Y. and ER Edelman, J Biomed Mater Res 1998;42:188-98. A seeding apparatus is used to allow simultaneous seeding of several grafts. A suitable seeding apparatus is described in: Mazzucotelli JP, Roudiere JL, Bemex F, Bertrand P, Leandri J, Loisanse D., Artif Organs 1993;17:787-90.

**EXAMPLE 3*****Flow Apparatus***

[0118] A suitable flow apparatus for use with the present invention is described in: Flugelman MY, Vinnani R. Leon MB, Bowman RL, Dichek DA. Circulation Research 1992;70:348-354. To be able to calculate the shear forces operating on the grafts some modifications from the original flow apparatus are performed in accordance with: Schneider A, Chandra M, Lazarovici G et al. Thromb Haemost 1997;78:1392-8.]

**Endothelial cell quantitative morphometry**

[0119] At the end of the incubation period the grafts are cut longitudinally, examined under fluorescent microscope, photographed and the images processed by an image analysis system.

**[t-PA and Urokinase measurements]**

[0120] t-PA level and activity are measured from supernatant collected from seeded grafts. t-PA and urokinase are tested by ELISA while t-PA activity is measured as described in Dichek DA, Nussbaum O, Degen SJF, Anderson WF, Blood 1991;77:533-41.]

**Cell recovery from grafts**

[0121] Cells are recovered from the PTFE grafts by EDTA-trypsin digestion. Cells are counted following digestion and re-plated in a tissue culture dish precoated with fibronectin to assess viability. A time from plating to full confluence is recorded. In cells transduced with pseudo-retroviral vectors VEGF and DANCE expression is measured.

**EXAMPLE [4] 3*****[Animal experiments]***

[0122] Short venous segment of sheep hind limb are used for endothelial cell harvesting. Cells are expanded in the laboratory, gene transfer with viral vectors is performed and the seeded grafts are implanted end-to-end proximally and distally into the carotid arteries of the donor sheep. A graft seeded with genetically modified cells is implanted in one side of a carotid artery, while a graft seeded with cells expressing only a marker gene is implanted in the other side of the carotid artery. Short-term and long-term experiments (3, 7, 30 days) are conducted to allow detection of cell adherence and local thrombosis and inflammation. Long term graft durability and flow will be assessed at 7, 30, and 180 days (n=5 for VEGF and n=5 for DANCE) by external measurements of flow using a Doppler flow meter (Transonic Animal Research Flowmeter, NY, USA). At the end of the follow up period, the grafts are retrieved and neointimal formation is assessed by morphometric measurements of cross sections while seeded cell adherence is tested by detection of marker gene expression.

[0123] Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All [publications,] patents[, patent applications and sequences] disclosed [therein] and/or nucleotide sequences identified by a Genbank accession number mentioned in this specification are herein incorporated in their entirety by reference [into the specification,] to the same extent as if each individual [publication,] patent[, patent application] or sequence was [specifically and individually indicated to be incorporated herein by reference] expressly set forth herein. All references to non-patent publications are provided as a convenience to the reader seeking additional information. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

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